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THERANOS, INC.

Ammonia Development Report [Plasma]

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Development Report
Ammonia Assay

1. Analyte Background[XE "Analyte Background"]

Ammonia is naturally produced in the body as a byproduct of bacterial digestion of proteins in the intestines. It is further converted to urea in the liver which is either used for protein synthesis or eliminated in urine. Higher levels of ammonia typically indicate the inability of the liver to function normally. Other reasons for high ammonia concentration include heart failure, kidney failure, severe bleeding from the stomach or intestines.

Analyte Range:

Reference ammonia ranges are 15-60 µg/dL for adults, 70-135 µg/dL for children and 170-340 µg/dL for newborns (Fischbach FT, Dunning MB III, 2009, Manual of Laboratory and Diagnostic Tests, 8th ed. Philadelphia: Lippincott Williams and Wilkins).

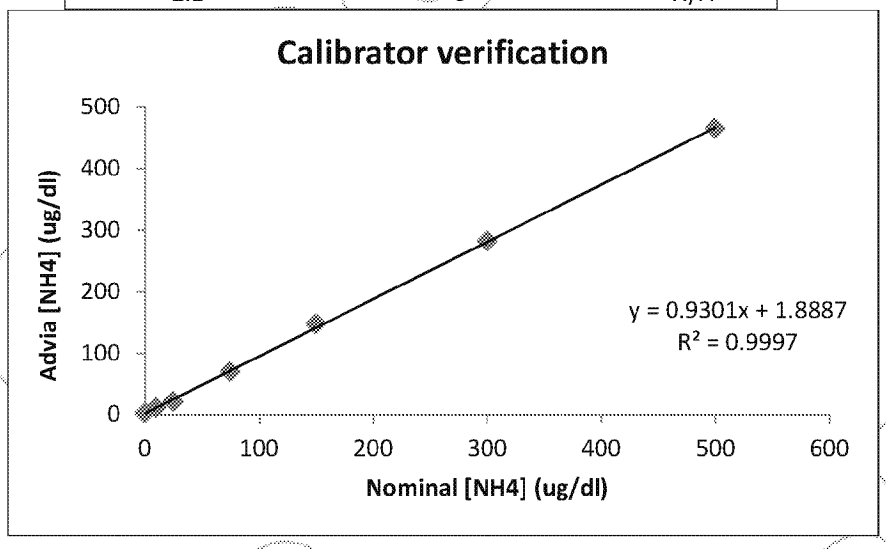
2. Reference Method[XE "Reference Assay"]

The ammonia assay provided with the Siemens Healthcare Advia 1800 was used as the reference method for the development of this assay.

3. Calibrator Verification

The Theranos calibrators were made by dissolving ammonium salt in water to make a high concentration stock which was then diluted into water to make 6 calibrator levels. These calibrators were verified by the Advia 1800 and showed good correlation between nominal and Advia-assigned values.

Advia [NH4] (µg/dl)	Nominal [NH4] (µg/dl)	%Recovery
465	500	93%
282	300	94%
147	150	98%
70	75	93%
22	25	88%
11	10	110%
2.1	0	N/A



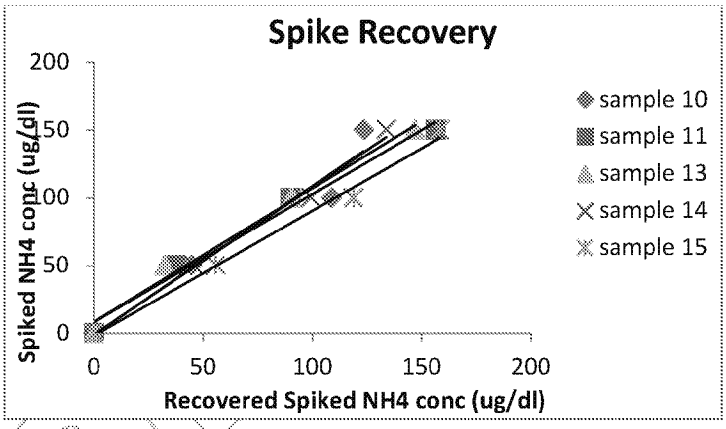
4. Spike Recovery

A spike recovery experiment was performed by spiking separate plasma samples with ammonium and testing each with the Theranos Ammonia Assay to determine the percent recovery. The spike recovery showed that there are no significant matrix affects with normal plasma. The average percent recovery across the spiked plasma sets was 95% and the average deviation between sets was only 12 µg/dL ammonia showing that ammonia concentrations can be accurately recovered in plasma samples.

	Spiked Ammonia Concentrations (ug/dL) Recovered							
Spiked NH4 (ug/dl)	Sample 10	Sample 12	Sample 13	Sample 14	Sample 15	Average	SD	CV
150	124	156	149	134	159	144	15	10%
100	109	90	91	99	119	102	12	12%
50	45	37	33	46	56	43	9	21%
0	0	0	0	0	0	0	0	-

Spiked NH4	Percent Spiked Ammonia Recovered	Average	SD	CV

(ug/dL)	Sample 10	Sample 12	Sample 13	Sample 14	Sample 15			
150	82%	104%	98%	89%	106%	96%	0.10	10%
100	109%	90%	91%	99%	119%	102%	0.12	12%
50	90%	74%	65%	91%	112%	86%	0.18	21%
0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A



5. Precision

In three separate experiments (freshly prepared reagents from stocks and freshly made plasma and calibrator dilutions) four unaltered plasma samples were tested for ammonia levels to show the intra- and inter-experiment precision of the assay. The average intra-run signal CV was 3% and the average inter-run signal CV was 4.2%. In terms of reported ammonia concentrations, the average intra-run precision was 3% and the average inter-run precision was 7% across three runs.

Signal

Sample#	Run 1	Run 2	Run 3	Average	SD	CV
4	0.149	0.158	0.154	0.154	0.0045	3%
6	0.126	0.136	0.136	0.133	0.0058	4%
8	0.130	0.139	0.139	0.136	0.0052	4%
10	0.188	0.208	0.208	0.201	0.0115	6%
				Average		4.2%

Calculated Concentrations

Sample#	Run 1	Run 2	Run 3	Average	STDV	% CV
4	739	765	809	771	35	5%
6	624	646	683	651	30	5%
8	642	662	783	696	76	11%
10	931	1010	1067	1003	68	7%
				Average	52	7%

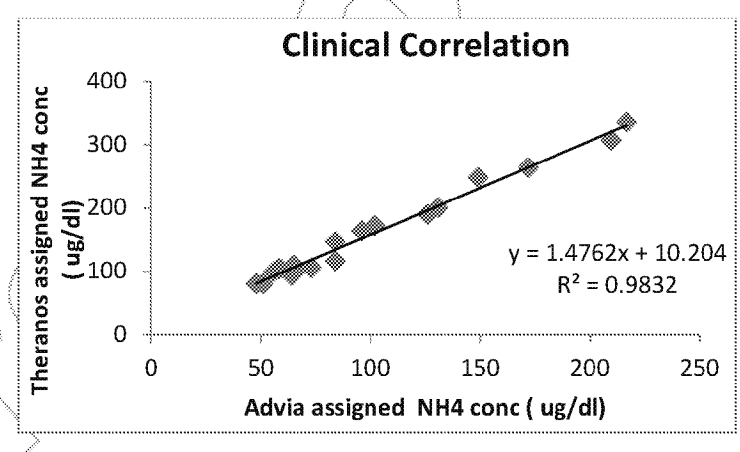
Inter-Run Concentration CVs

Sample#	Run 1	Run 2	Run 3	Average
4	2%	1%	4%	2%
6	7%	4%	1%	4%

8	1%	0%	7%	3%
10	2%	5%	1%	3%
Average	**3%**	**3%**	**3%**	**3%**

6. Clinical Correlation

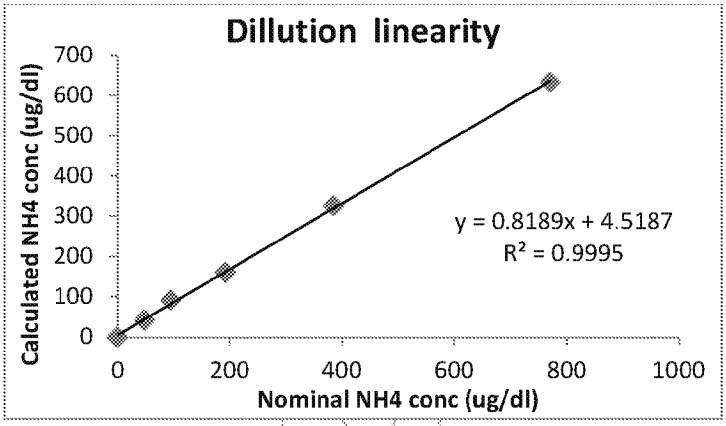
Twenty spiked plasma samples covering the clinically-relevant range were testing by the Theranos Ammonia Assay and by the Advia 1800. The resulting ammonia concentrations for each sample were plotted to show the correlation of results between the two methods. The resulting correlation provides a slope of 1.4 with $R^2 = 0.98$.



7. Linearity[XE "Dilution Linearity"]

To confirm that the assay response is linear with respect to the concentration of ammonia present in a plasma sample, a dilution linearity test was run. A plasma sample with a high endogenous level of was serially diluted and assessed using the Theranos Ammonia Assay. Linear regression analysis showed that the assay is linear from 0-600 µg/dL with $R^2 = 0.999$.

Nominal [NH4] (µg/dL)	Calculated [NH4] (µg/dL)	Conc. SD	Conc. %CV	%Recovery
771	633	5	1%	82%
386	326	38	9%	84%
193	159	2	1%	83%
96	92	17	8%	96%
48	40	9	6%	84%
0	0	1	1%	N/A



8. Interference[XE "Interference"]

Interference due to icteric, lipemic, or hemolyzed samples was tested by spiking such samples with ammonia and measuring the percent spike recovery across three levels spanning the range of diagnostic interest. The lipemic and icteric samples gave concentration recovery within 4% showing no recovery interference. Higher concentration lipemic result in under-recovery for all three levels of spiked ammonia. Ammonia was recovered from mildly hemolytic samples at 94%, on average. The ammonia assay is not effective for measuring ammonia in very hemolytic samples since the ammonia recovered was only 61%. This shows that high concentrations of triglycerides and high concentrations of hemoglobin in plasma result in a matrix affect.

Reported Ammonia Spike Concentration (µg/dL)

Spiked[NH4] (µg/dL)	Icteric Low	Icteric High	Lipemic Low	Lipemic Low	Lipimic High	Hemolyzed Low	Hemolyzed High
150	125	131	131	131	118	130	102
100	82	90	98	100	38	95	72
30	25	26	38	38	19	30	13
0	0	0	0	0	0	0	0

Reported Ammonia Percent Recovery (µg/dL)

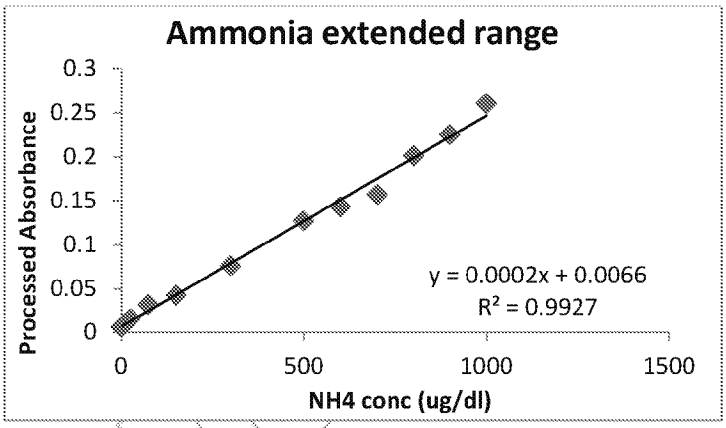
Spiked[NH4] (µg/dL)	Icteric Low	Icteric High	Lipemic Low	Lipemic Low	Lipemic High	Hemolyzed Low	Hemolyzed High
150	83%	87%	87%	87%	79%	87%	68%
100	82%	90%	98%	100%	38%	95%	72%
30	98%	87%	128%	111%	63%	101%	43%
Total recovery	**88%**	**88%**	**104%**	**99%**	**60%**	**94%**	**61%**

9. Extended Range Testing[XE "ULOQ and LLOQ"]

The high limit of detection from this assay was determined by testing high concentrations of ammonia up to 1000 µg/dL. The lower limit of detection is set by the smallest calibrator concentration of 10 µg/dL ammonia.

Ammonia dissolved in water was used to make the high ammonia levels. The upper limit of detection was found at 1000 µg/dL which is much higher than the clinical range.

Therefore, the overall range of the assay is 0-1000 µg/dL which sufficiently covers the whole clinical range.



10. Stability

Stability studies for the Theranos Ammonia Assay are ongoing.

11. Conclusions[XE "Conclusions"]

The Theranos Ammonia assay has completed development testing and met the necessary testing criteria. Acceptable precision, accuracy, and response have been demonstrated.